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Ontogeny of the Barley Plant as Related to Mutation Expression and Detection of Pollen Mutations

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Clustering of mutant pollen grains in a population of normal pollen due to premeiotic mutational events complicates translating mutation frequencies into rates. Embryo ontogeny in barley will be described and used to illustrate the formation of such mutant clusters. The nature of the statistics for mutation frequency will be described from a study of the reversion frequencies of various waxy mutants in barley.

Computer analysis by a "jackknife" method of the reversion frequencies of a waxy mutant treated with the mutagen sodium azide showed a significantly higher reversion frequency than untreated material. Problems of the computer analysis suggest a better experimental design for pollen mutation experiments. Preliminary work on computer modeling for pollen development and mutation will be described.

The determination of mutation frequencies in pollen systems produces many technical problems such as consistency in mutation scoring, accurate counting of pollen grains, staining procedures, and eventually automation of these procedures. However, before the mutation frequencies can be translated into mutation rates, the different factors that influence pollen mutation frequencies must be understood.

Typical pollen mutation data, which in this case are spontaneous reversion data of an azide-induced waxy mutant (Az 22) in barley, are shown in Table 1. These data are from the analysis of spikes from four plants. The individual spikes show almost a twentyfold difference in reversion frequency from the highest to the lowest. Some of the spikes show pronounced mutant clustering, such as spike #1 from plant 80-42 where the reversion frequency is nearly 10^{-3}.

Although spike reversion frequencies show considerable variability, reversion frequencies for whole plants can be quite homogeneous. The reversion frequencies for the plants in Table 1 were 3.16×10^{-4}, 1.64×10^{-4}, 1.69×10^{-4}, and 1.75×10^{-4}, respectively.

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The developmental phase of the plant at the time of mutation induction has a great effect on mutant cluster size. The ontogeny in the gramineae is well understood and will be used as an example to illustrate this effect. Studies of early embryogenesis in the grasses show that the early embryo is undifferentiated up to the seventh day after fertilization in corn (*1*). When the seed is developed completely, there is a root and a shoot system with 3-5 leaves. Mullenax (*2*), in a study of the mature barley seed, showed the differentiated leaves in the seed, and at this stage there were two tiller buds differentiated, one in the axil of the coleoptile and the other in the axil of the first leaf. The tiller buds are of great importance since each may initiate an inflorescence. By the seventh day following germination in barley, as many as six primary tillers and a number of secondary tillers can be initiated. Each tiller which develops will produce a mature spike with up to 60 florets which contain 180 anthers and 250,000 or more pollen grains.

Within each spike during the maturation process there is a wide range of floret development. For pollen mutagenesis it is very important to understand the inflorescence development since the timing of the mutagen treatment in respect to the development of the inflorescence will affect the

Table 1. Reversion frequencies of waxy mutant Az 22.

Plant no.	Spike no.	No. pollen	No. revertant pollen	Reversion frequency
80-42	1	48,200	47	9.75×10^{-4}
	2	30,200	20	6.62×10^{-4}
	3	70,520	12	1.70×10^{-4}
	4	78,360	10	1.28×10^{-4}
	5	91,880	12	1.31×10^{-4}
80-43	1	55,600	15	2.70×10^{-4}
	2	45,240	7	1.55×10^{-4}
	3	52,040	3	5.76×10^{-5}
80-44	1	46,880	15	3.20×10^{-4}
	2	115,840	17	1.47×10^{-4}
	3	18,120	8	4.42×10^{-4}
	4	69,560	6	8.62×10^{-5}
	5	48,040	3	6.24×10^{-5}
	6	50,600	10	1.98×10^{-4}
80-45	1	34,160	6	1.75×10^{-4}

mutation frequency data. Williams (*3*) grew wheat plants under uniform conditions in order to study the events following seed germination. At the 16th day of growth, the sixth leaf was barely 0.4 mm in length, but at this stage the apex was elongating prior to spike formation. From this time on spikelet initials grow precociously and foliar growth is restricted. In a typical spike, by the 25th day all the spikelets are induced, and by 30 days the first stamens appear. Both stamens and carpels are developed by 34 days and the florets in the spike are essentially complete by 50 days, with anthesis at the 55th day following germination.

Tillering in barley and wheat is a further complicating factor. The first tiller primordium in the axil of the coleoptile usually does not develop, but the other tillers potentially will develop and show a wide range of stages of spike development at any one time. Obviously, mutation frequency data will be influenced greatly by the tiller sampled. A good sampling procedure is to take the first primary spike from each plant in a plot.

There are a number of reasons for mutant clustering and the developmental phase of the plant at the time of the mutational event is an important one. A mutation in a tiller initial in a seed would yield a large cluster of mutant pollen grains, whereas a mutation at meiosis would theoretically yield one or two mutant pollen grains. Clustering can also be caused by persistence of mutagens and/or coincidence of mutation (*4*).

Another factor influencing mutant cluster size is the so-called genetically effective cell number (*5*). This GECN is essentially the number of primordial cells at the time of mutation induction which develop into an inflorescence. A careful study of chlorophyll mutation segregation ratios in primary barley spikes in our laboratory shows that their average GECN is six. Chlorophyll mutation segregation ratios for individual spikes appear approximately in a series of 3:1, 7:1, 11:1, 15:1, etc. Table 2 shows the relationship between GECN and segregation ratios.

Individual spikes scored vary in apparent GECN from one to twelve or more; however, the concept is probably valid only as a gross average since there are so many other factors affecting segregation ratios. The GECN determined for barley from M_3 data by the method of Li and Rédei (*5*) also gave a value of six.

The most difficult statistical problem in deriving mutation rates from mutation frequencies is estimating the number of mutational events which produce a mutational cluster. The cluster could be caused by one mutation early in the plant development or a number of events later in the development (*6*). The type of mutagen is of importance also, since a persistent mutagen such as azide would be expected to produce more coincidence of mutation than mutagens such as radiation.

Table 2. Genetically effective cell number (GECN)

Segregation ratio	GECN
3:1	1
7:1	2
11:1	3
15:1	4
19:1	5
23:1	6
27:1	7
31:1	8
35:1	9
39:1	10
43:1	11
47:1	12

There have been a number of methods for analysis of mutation data (*6-9*). The statistical methods are needed to give a test of significance between mutation frequencies of treated materials and controls and also differences between the spontaneous reversion frequencies of various waxy mutants. Computer analysis of our data by one of the present authors (*10*) showed that the models generally used for mutational data are too simplistic. The statistical distributions such as binomial, Poisson, or negative binomial were not appropriate for the data. A "jackknife" method was then used to analyze the data (*11*). This is a relatively model free data-based method that yields an estimate of the variability. The jackknife starts with the easiest estimate which is the number of revertants divided by the number of pollen grains in that spike. These are the statistics derived: p = the true proportion of mutations, $\hat{P}_{ij}$ is the raw estimate = X_{ij}/N_{ij} in spike i of plant j, where X = number of revertants and N = total number of pollen; $\mu = -\log(L - P)$ = mean rate of mutation initiation, the raw estimate is $\hat{\mu}_{ij}$; $Q = \log \mu$; and the estimate is $\hat{Q}_{ij}$ is the logarithm of the rate of initiation of mutations estimated for each spike. The log transformation can be justified as being nearly "variance stabilizing."

A grand mean of the p estimates for each spike is computed, and then the data are recomputed sequentially, leaving out one spike at a time, and a series of so-called pseudo estimates of Q are derived which are the natural logarithms of the rate of mutation initiation. In the next step the average of pseudo estimates is compared with the grand mean. A relatively model free standard error of the mean can be computed by using the pseudo estimates.

The jackknife method was used on some preliminary 1979 waxy mutant data. The difference between mutagen-treated and untreated Az 22 reversion frequency was analyzed, and although the variation between plants was great, a significant difference in reversion frequency was demonstrated.

The 1980 data were much more consistent due to better revertant scoring and sampling methods. As a result of this analysis of azide-induced reversions, four waxy mutants are being studied in more detail. For this study two putative γ-ray-induced waxy mutants and two azide-induced mutants have been chosen. The two gamma mutants differ significantly in reversion frequencies, as do the two azide mutants. Four different azide concentrations will be used constituting a test of the computer method to differentiate treatment effects and a test of the sensitivity of the waxy mutants to small differences in mutagen concentration. The optimum plot sampling method has not been worked out; however, it is clear that a sample of at least five to ten plants and four or more spikes per plant giving a total pollen number scored for mutations of over a million is the minimum necessary.

A computer model for azide mutagenesis in barley is being attempted. In a simplified approach we have included the factors that the mutagen is persistent from seed treatment until meiosis, that the mutagen is diluted by the cell number increase, and that there is a tenfold sensitivity increase during meiosis (*9*). Starting from this simplistic approach, it is hoped that we will be able to simulate the data from actual mutant counts, and in this way produce a good model for analysis of the pollen mutation data.

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REFERENCES

1. Wardlaw, C. W. Embryogenesis in Plants. John Wiley and Sons, Inc., New York, 1955, pp. 270-278.
2. Mullenax, R. H. Dormant embryo morphology, seedling morphogenesis and tiller development of Himalaya barley. Washington Agricultural Experimental Station Technical Bulletin 55, Washington State University, Pullman, 1967.
3. Williams, R. F. The Shoot Apex and Leaf Growth. Cambridge University Press, Cambridge, 1974.
4. Lindgren, D., Eriksson, G., and Šulovská, K. The size and appearance of the mutated sector in barley spikes. Hereditas 65: 107 (1970).
5. Li, S. L., and Rédei, G. P. Estimation of mutation rate in autogamous diploids. Radiat. Bot. 9: 125 (1969).
6. Engels, W. R. The estimation of mutation rates when premeiotic events are involved. Environ. Mutagen. 1: 37 (1979).
7. Katz, A. J. Design and analysis of experiments on mutagenicity. I. Minimal sample sizes. Mutat. Res. 50: 301 (1978).
8. Katz, A. J. Design and analysis of experiments on mutagenicity. II. Assays involving microorganisms. Mutat. Res. 64: 61 (1979).
9. Lindgren, D. Sensitivity of premeiotic and meiotic stages to spontaneous and induced mutations in barley and maize. Hereditas 79: 227 (1975).
10. Marcus, A. H. Personal communication.
11. Gladen, B. The use of the jackknife to estimate proportions from toxicological data in the presence of litter effects. J. Am. Statist. Assoc. 74: 278 (1979).